PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

CORRECTED VERSION

Applicant's or agent's file reference 512 WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/EP 03/50097	International filing date *(day/month/year)* 09.04.2003	Priority date *(day/month/year)* 10.04.2002
International Patent Classification (IPC) or both national classification and IPC C07K14/52		
Applicant APPLIED RESEARCH SYSTEMS ARS HOLDING N.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

I	☒	Basis of the opinion
II	☐	Priority
III	☒	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☐	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
25.09.2003	18.05.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Trommsdorff, M Telephone No. +49 89 2399-7361

CORRECTED VERSION

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/50097

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-44 as originally filed

Claims, Numbers

1-24 received on 15.12.2003 with letter of 11.12.2003

Drawings, Sheets

1-11 as originally filed

Sequence listing part of the description, pages:

1-6, filed with the letter of 01.07.03,

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☒ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readable form.

   ☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 17-20, 22-24

   because:

   ☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-10, 16-24
	No:	Claims	11-15
Inventive step (IS)	Yes:	Claims	2, 6
	No:	Claims	1, 3-5, 7-24
Industrial applicability (IA)	Yes:	Claims	1-16, 21
	No:	Claims	17-20, 22-24

2. Citations and explanations

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/50097

see separate sheet

1. Cited documents

D1: HEMMERICH STEFAN ET AL: 'Identification of residues in the monocyte chemotactic protein-1 that contact the MCP-1 receptor, CCR2' BIOCHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, PA, US, vol. 38, no. 40, 5 October 1999 (1999-10-05), pages 13013-13025,ISSN: 0006-2960 cited in the application

D2: STEITZ S A ET AL: 'Mapping of MCP-1 functional domains by peptide analysis and site-directed mutagenesis' FEBS LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 430, no. 3, 3 July 1998 (1998-07-03), pages 158-164, ISSN: 0014-5793 cited in the application

D3: SEET BRUCE T ET AL: 'Molecular determinants for CC-chemokine recognition by a poxvirus CC-chemokine inhibitor.' PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES, vol. 98, no. 16, 31 July 2001 (2001-07-31), pages 9008-9013, July 31, 2001 ISSN: 0027-8424 cited in the application

2. Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

For the assessment of the present claims 17-20 and 22-24 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

3. Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

3.1. Claim 1 is directed to antagonists of MCP proteins containing mutations at specific positions.

D1 discloses mutants of MCP-1: all surface exposed residues were mutated with

alanine. R18A and K19A mutants are disclosed as well as K44A and K58A mutants. Mutants with several mutations are also disclosed as, e.g. [1+10-76, 7/9] with among others mutations in K19 and K44 and K58. The binding affinity of mutants in positions 18 and 19 is 2-3 fold decreased (p.13016, right column). Since the mutants of D1 which are mutated in the positions partly claimed in claim 1 do not show an antagonistic effect, the subject-matter of claim 1 and dependent claims 2-10 and 15-24 can be considered to be novel (Art. 33(1) PCT).
D1 however clearly shows that mutations in positions 18 or 19 alone are not sufficient to yield antagonists (see Figure 3). Since in the application the antagonistic effect is also only shown for double mutants in positions 18 and 19, claim 1 should be restricted to mutants which contain at least mutations in said two positions.

3.2. Claim 11 is directed to peptide mimetics designed on the sequence and/or structure of the claimed antagonists. The scope of said claim is so broad that known peptides such as eg. those disclosed in D1 are prejudicial to the novelty of said claim (Art. 33(2) PCT).

3.3. Claim 12 is directed to DNA molecules encoding said antagonists "including nucleotide sequences substantially the same". This vague expression renders the scope of claim 12 so broad that known sequences disclosed in D1 fall into the scope of said claim and dependent claims 13-15 (Art. 33(2) PCT).

3.4. D2 uses site directed mutagenesis of MCP-1 to analyse the different functional domains of MCP-1. The mutants are tested for their activity. Mutant R18A shows similar activity to wild-type whereas Y13A mutant shows almost no activity. D2 further points to the important therapeutic consequences the identification of potent antagonists of MCP-1 would have (p.163, last paragraph).
In D3 MCP-1 mutants are analysed to define which amino acids are important for the interaction with the poxvirus CC-chemokine. The effect of such mutations on the binding affinity to the receptor CCR2b is also analysed. Residues 18 and 19 are found to be crucial residues for the interaction with VV-35kDa but much less for the binding to CCR2b (p.9011, 1st paragraph).
D3 also suggests that the determination of important structural features for the interaction between chemokines and their receptors will help to develop antagonists (p.9013, last paragraph).

The difference between claim 1 and D2 and/or D3 is that the mutants disclosed in said documents (which bear only single mutations in positions 18 or 19) do not show potent antagonistic properties.

The problem to be solved can be considered as the identification of more potent antagonists.

The problem is only partly solved, since the applicants show an antagonistic effect only for the double mutant in positions 18 and 19. For all the other mutants with mutations in only one of the two positions 18 or 19 in combination with other positions, no results are shown.

D1 shows however that not all mutants which fall into the scope of claim 1 are indeed antagonists (see [1+10-76, 7/9] with among others mutations in K19 and K44 and K58). This is further confirmed by the results obtained with single amino acid mutants disclosed in D2 and D3.

Thus, only mutants with at least mutations in positions 18 and 19 as claimed in claims 2 and 6 can be considered as inventive over the prior art.

Hence, the subject-matter of claims 1 and dependent or related claims 3-5, 7-24 lacks an inventive step (Art. 33(3) PCT).

CLAIMS

1. Antagonists of MCP proteins consisting of mutants of MCP proteins in which the following combinations of residues, numbered on the sequence of human mature MCP-1, are substituted to Alanine, Glycine, Serine, Threonine, Proline, Aspartic acid, Asparagine, Glutamic acid, or Glutamine:

   a) a) 18 and 19;

   b) 18 and/or 19, together with 66;

   c) 18 and/or 19, together with 58 and 66;

   d) 18 and/or 19, together with one or more of the following: 24, 44, 49, 75.

2. The antagonist of claim 1 wherein residues 18 and 19 are substituted with Alanine.

3. The antagonist of any of the claims from 1 or 2 wherein the MCP proteins are human MCP-1, human MCP-2, human MCP-3, human MCP-4, or human Eotaxin.

4. The antagonist of any of the claims from 1 or 2 wherein the MCP proteins are proteins having at least 70% of homology with human mature MCP-1, MCP-2, MCP-3, MCP-4, or Eotaxin.

5. Active mutants of the antagonists of MCP proteins of any of the claims from 1 to 4, in which one or more amino acid residues have been added, deleted, or substituted without interfering with the antagonistic activity.

6. The antagonist of claim 5, wherein the polypeptide has the sequence corresponding to SEQ ID NO: 3.

7. Antagonist of MCP proteins comprising the amino acid sequence of any of the claims from 1 to 6, and an amino acid sequence belonging to a protein sequence other than the corresponding MCP protein.

8. The antagonist of claim 7, wherein the amino acid sequence belonging to a protein sequence other than the corresponding MCP protein is an amino acid sequence belonging to one or more of these protein sequences: extracellular domains of membrane-bound protein, immunoglobulin constant region, multimerization domains, extracellular proteins, signal peptide-containing proteins, export signal-containing proteins.

9. The antagonist of any of the claims from 1 to 10, wherein said polypeptides are in the form of active fractions, precursors, salts, or derivatives.

10. The antagonist of any of the claims from 1 to 8, wherein said antagonist is in the form of active conjugate or complex with a molecule chosen amongst radioactive labels, biotin, fluorescent labels, cytotoxic agents, drug delivery agents.

11. Peptide mimetics designed on the sequence and/or the structure of the antagonists of MCP proteins of any of the claims from 1 to 6.

12. DNA molecules comprising the DNA sequences coding for the MCP antagonists of any of the claims from 1 to 8, including nucleotide sequences substantially the same.

13. Expression vectors comprising the DNA molecules of claim 12.

14. Host cells transformed with vectors of claim 13.

15. Process of preparation of MCP antagonists of any of the claims from 1 to 10, comprising culturing the transformed cells of claim 14 and collecting the expressed proteins.

16. Purified preparations of MCP antagonists of any of the claims from 1 to 10.

17. Use of MCP antagonists of any of the claims from 1 to 10 as medicaments.

18. Use of the MCP antagonists of claims from 1 to 10 as active ingredients in pharmaceutical compositions for the treatment or prevention of diseases related to excessive leukocyte migration and activation.

19. The use of claim 18 wherein the disease is an inflammatory disease, an autoimmune disease or an infection.

20. Use of the MCP antagonists of claims from 1 to 10 as active ingredients in pharmaceutical compositions for the treatment or prevention of vascular disorders or cancer.

21. Pharmaceutical composition containing a MCP antagonist of claims from 1 to 10 as active ingredient.

22. Method for the treatment or prevention of diseases related to excessive leukocyte migration and activation, comprising the administration of an effective amount of an MCP antagonist of claims from 1 to 10.

23. The method of claim 22 wherein the disease is an inflammatory disease, an autoimmune disease or an infection.

24. Method for the treatment or prevention of vascular disorders or cancer, comprising the administration of an effective amount of an MCP antagonist of claims from 1 to 10.